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Interaction of antibodies with renal cell surface antigens

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Over the past ten years it has been increasingly recognized that immune complex nephritides, characterized by the accumulation of granular immune deposits in renal structures, may not only be the consequence of deposition of circulating immune complexes but also of antigen-antibody complexes aggregated locally or formed in situ [1]. One of the sites at which antibodies may interact locally with antigens in the kidney is at the surface of renal cells [1, 2]. There is an abundance of antigens on cells: part of them are intrinsic plasma membrane proteins, while others may be present because they are secretion products or they have been "planted" on the cell surface for immunological or physico-chemical reasons. In this article renal lesions that may arise from the binding of antibodies with these various kinds of cell surface antigens will be reviewed. Most of our insights come from animal models of renal disease. In the closing section the potential impact of data derived from experimental pathology on our concepts of human nephropathies will be evaluated.

Plasma membrane antigens

At the core of the membrane surrounding the plasm of cells is a bilayer of lipid molecules. A key property of this lipid bilayer is that, while being a stable planar structure, it possesses fluidity: proteins and lipids floating in the plasma membrane are able to move and diffuse laterally in any direction. The many kinds of proteins inserted into the plasma membrane function as receptors for a variety of ligands in the extracellular medium, form specialized transmembrane pumps or channels, convey a negative charge to the cell surface, or serve other not always yet defined physiological roles [2–4].

A few substances such as water and dissolved gasses may diffuse across the plasma membrane. For other substances in the extracellular milieu there are two principal routes into cells. The first is represented by the pumps and channels that transport ions and small water-soluble molecules such as amino acids and sugars into the cytoplasm. The second route, allowing large molecules and particulate matter to enter the cell, is by means of endocytosis. Three types of endocytosis are discerned. Very large particles may be brought into cells by phagocytosis. Fluid with whatever molecules happen to be in it may reach the cytoplasm by the process of pinocytosis. The third type, receptor-mediated endocytosis, involves the binding of ligands to their corresponding transmembrane (amphipathic) protein receptors and is, in contrast to phagocytosis and pinocytosis, very specific [3, 4]. A great variety of receptors has been described including specific receptors for transport proteins that deliver nutrients to cells (such as, low density lipoprotein, transferrin, ferritin), non-transport plasma proteins (such as, α-$_2$ macroglobulin, immune complexes), cytokines (such as, protein growth factors, interleukins), hormones (such as, insulin, luteinizing hormone), certain enzymes, toxins and lectins, and viruses [3–5]. Receptors (cell surface Ig and MHC antigens) also play a pivotal role in the immune response [3, 6–8]. The receptor makeup is different from one cell type to another. Receptor-mediated endocytosis is initiated by ligands joining their respective receptors. If not already inside coated pits, receptor-ligand complexes move laterally to cluster in these specialized plasma membrane structures. Coated pits, found on all cells except red blood cells, are so named because these plasma membrane indentations are covered at the cytoplasmic side by a furry coat mainly composed of a characteristic protein, clathrin [9]. Coated pits are short-lived as they, with different receptor-ligand complexes inside, in an energy-dependent process, pinch off the plasma membrane to form intracellular coated vesicles. Once inside the cell, the vesicles shed their clathrin coat, fuse with one another and become part of the endosomal compartment, which is composed of a network of vesicles and tubules [3, 4, 10]. Helped by a low pH in the endosomal compartment, many types of ligands disconnect from their receptors to be dispatched to lysosomes where they are degraded. By contrast, the membrane lipids together with receptors rejoin the plasma membrane in a process of exocytosis [3, 4, 10]. One of the alternative final destinations of ligands is the release from the cell at a site other than that of entrance. This has been shown for example for maternal IgG in newborn rats and for secretory IgA [11]. The process of endocytosis then entails a rapid sequence of intricate and complex rearrangements and fusion of membranes with some types of cells internalizing as much as half of the plasma membrane per hour. Whereas most types of receptors are internalized in coated pits only, a notable exception is the insulin receptor, which may also be endocytosed, in a non-concentrative manner, in pinocytotic invaginations [12]. One might expect that lack of functional receptors could lead to disease. In fact this has been convincingly demonstrated in patients with familial hypercholesterolemia [3, 10].

While most natural ligands will not crosslink their plasma membrane receptors, such a situation can conveniently be created and studied in vitro by using antibodies against such diverse receptors as surface Ig, thymus leukemia antigen, measles hemagglutinating antigen, and Fc and C3b/C4b recep-

tors [2, 5, 13–17]. The rapid succession of changes following the binding of antibodies to these receptors may be divided into three phases [5, 13]. First, the receptors cluster in small patches that by diffusing laterally fuse into larger ones (micro-immune complex-precipitates). This is a passive process requiring only divalency of the antibody and a temperature above 4°C. In the second phase, the patches accumulate in a cap at one pole of the cell. This antibody-induced receptor redistribution is associated with increased cell motility. With the leading, "ruffled" edge of the motile cell free from receptor-antibody complexes, these complexes are concentrated in the cap at the rear of the cell (uropod). This phase is energy-dependent. Thus capping is prevented not only by low temperature but also by inhibitors of the respiratory chain or of glycolysis. Another requirement for capping is an intact, functional cytoskeleton. The cytoskeleton ("the muscles and bones of the cell") is composed of microfilaments (also referred to as actin filaments), intermediate filaments, microtubules, and nuclear structures [18]. Although the precise mechanism is unknown, crosslinked transmembranous proteins connect with the microfilaments and these structures together with myosin can be seen to aggregate in the cytoplasm underneath the caps [13, 19, 20]. Further support for the participation of the cytoskeleton comes from the observation that drugs that interfere with cytoskeletal function (cytochalasin B, phenothiazines) also interfere with cap formation [21, 22]. In the third phase the caps are removed by endocytosis and, in some instances at least, by shedding, leaving the cell surface devoid of the receptors involved. This phenomenon has been called "antigenic modulation", as antibodies will no longer find their targets on the plasma membrane [5, 14]. In the absence of further exposure of the cells to specific antibodies, the surface receptors are most of the time reexpressed. All three phases of the antibody-mediated receptor redistribution take place in a matter of minutes.

Plasma membrane antigens that upon crosslinking do not become anchored to the cytoskeleton, as for example Thy-1 and MHC Class I antigens and certain glycolipids still can be induced to cap [6, 13, 17, 23–26]. This capping reaction called "passive" to contrast with the active process described above, has much slower kinetics, may require a double layer of ligands, and is not inhibited by cytoskeleton-suppressing drugs [13, 17]. It may be that in passive capping, directional plasma membrane recycling is a driving force [26]. This latter mechanism may also be invoked to explain some degree of redistribution of surface antigens occurring spontaneously in moving cells or in cells forming a uropod [26, 27].

Several studies have addressed antibody-mediated plasma membrane antigen redistribution in vivo [reviewed in 5]. In one such study [28] for four days rabbits received goat antibodies directed against angiotensin converting enzyme (ACE), a transmembrane protein especially expressed on the surface of alveolar endothelial cells [29]. Due to pulmonary edema, many animals did not survive the first day of injection. Immunofluorescence microscopy revealed granular deposits (patches) of goat IgG, rabbit C3, and ACE along the alveolar capillary endothelium. The granular distribution of ACE after interaction in vivo with antibody contrasted with the even, linear expression of the enzyme on the surface of the alveolar endothelium observed in normal lung. Rabbits surviving the first day tolerated subsequent administration of anti-ACE antibodies without clinically apparent ill effects. The change in the response to antibody injection was explained by the disappearance of ACE from the lung after continued interaction with specific antibody. The "immunological enzymectomy" of ACE from the lung was interpreted as an example of antigenic modulation. Within 24 hours of discontinuation of antibody administration, ACE was detectable again in the lung.

As stated before, the capped receptor-antibody complexes are removed by endocytosis [13, 30]. To what extent shedding of the complexes contributes to the removal process is in most instances unknown [5, 31]. In at least one system, involving oocytes, there is convincing evidence that shedding does occur [32]. The first description of the coated pit system and the correct interpretation of its function came from studies on insect eggs [33]. It was recognized that yolk proteins produced elsewhere enter the oocyte via coated pit-directed endocytosis. In rabbit oocytes, ACE appears to be present as a surface protein [34]. The expression of ACE is diffuse and not limited to coated pits. Binding in vivo of goat anti-ACE antibodies to ACE induces redistribution of ACE followed by the appearance in the zona pellucida of morphologically recognizable aggregates composed of goat IgG, rabbit C3, and ACE. The shedding and aggregation of immune complexes seems to take place preferentially at the level of the oocyte microvilli, a situation resembling that observed for crosslinked Ig on B-cells [27]. Small round profiles noted around and in the immune deposits may be oolemma-derived [32].

A physiological role for crosslinkage of plasma membrane proteins is illustrated by B-cell differentiation, proliferation, and antibody secretion subsequent to binding of polyvalent antigens to surface Ig [13, 35, 36]. Plasma membrane redistribution and antigenic modulation may also be important for understanding pathological conditions, among them the renal diseases discussed below in this section, certain autoimmune diseases, and chronic viral infections [5, 15, 37]. Lastly, antigenic modulation should be taken into account in therapeutic modalities involving the use of antibodies against cell surface antigens [5].

Glomerular visceral epithelial cells

1. Heymann glomerulonephritis. a. Introduction. In 1959 Heymann et al [38] demonstrated that active immunization of rats with whole rat kidney homogenates incorporated into Freund's adjuvants induces a nephrotic syndrome. Proteinuria results from abnormal deposits of immunoglobulins and complement in the glomerular capillary walls, corresponding to deposits of foreign material at the epithelial side of the basement membrane [39]. These findings are similar to those seen in the glomeruli of patients with idiopathic membranous glomerulonephritis, and Heymann glomerulonephritis has been accepted as a model for the human disease. Edgington, Glassock and Dixon [40] and Grupe and Kaplan [41] demonstrated that the antigen is localized in a membrane preparation (Fx1A) derived from rat proximal convoluted tubules. A more purified and nephritogenic fraction was called RTEα5 [42]. Miettinen et al used lectin affinity chromatography to isolate eight glycopeptides. This was the first indication that the antigen is a glycoprotein [43]. Because antibodies eluted from glomeruli of rats with Heymann glomerulonephritis react with glomeruli of nephritic, but not of normal rats, the disease was considered as

due to a local deposition of immune complexes formed in the circulation by a tubular brush border antigen and corresponding antibody [42]. This interpretation was strengthened by the first descriptions of passive Heymann glomerulonephritis. When it was shown that rats injected with heterologous [44, 45] or autologous [46] brush border antibodies rapidly develop Heymann glomerulonephritis, the lesions were again ascribed to circulating immune complexes because the available immunohistological techniques did not detect the binding of antibody to normal glomeruli in tissue sections. The challenge to this basic concept originated from the studies of van Damme et al [47] and Couser et al [48] who, using ex vivo or isolated perfused kidneys, showed that the anti-Fx1A antibodies fix directly to glomeruli. The results indicated that the antigen is present in the glomerular capillary wall and is similar enough to that on the tubular brush border to be recognized by the antibody. The visualization of the glomerular antigen in vitro was more difficult to achieve. Bertani et al [49] described a weak binding of heterologous affinity-purified Fx1A antibody to glomeruli in tissue sections. Similar results were reported by Neale et al [50], who used antibodies eluted from the kidneys of rats with Heymann glomerulonephritis. However, the definitive evidence that the most relevant antigen of Heymann glomerulonephritis is expressed at the surface of glomerular visceral epithelial cells, its partial biochemical characterization, and the demonstration of its nephritogenicity were provided by Kerjaschki and Farquhar [51, 52]. Their results stimulated the search for other structural nephritogenic antigens in the rat, in other laboratory animals and in man. Moreover, attention was focused on the pathogenic role of Heymann antibodies in the epithelial cells of proximal tubules and of the yolk sac.

b. Antigens. The development of monoclonal antibody technology and the application of plasma membrane biochemistry to the study of glomerular diseases allowed to identify several antigens at the surface of tubular and glomerular cells. The membrane composition of the epithelial cells in the initial (S_1 and S_2) segments of the proximal tubule is characterized by distinct microdomains [53]. Maltase is concentrated in the microvilli and similarly distributed are the 130 and 94 gamma-glutamyl transpeptidases and the 70-90 kD dipeptidyl peptidase IV. The latter is also present in the Golgi apparatus, in intracytoplasmic vacuoles and on the basolateral membranes [54]. gp330 is a glycoprotein of 330 kD which was isolated from purified rat brush border fractions, solubilized in detergent, iodinated and subjected to precipitation with IgG eluted from glomeruli of rats with Heymann glomerulonephritis. The protein was subsequently purified by gel filtration and lentil lectin affinity chromatography [51]. gp330 is concentrated in clathrin-coated apical invaginations at the base of the microvilli [53]. In normal rats gp330 is not internalized during endocytosis, whereas, in rats treated with sodium maleate, which reduces protein reabsorption, it is internalized in newly formed apical vacuoles. The lysosomal membrane glycoprotein, lgp120, is restricted to lysosomes, whereas endosomes do not contain any of the antigens previously mentioned [55]. Since Heymann antibodies are present in the glomerular filtrate of proteinuric rats it is not surprising that tubular injury may develop (see section Tubular cells: Heymann nephritis).

The best characterized glomerular antigen is gp330. By immunohistochemistry gp330 has been demonstrated in the Golgi elements, in the endoplasmic reticulum, in the multivesicular bodies and in the coated pits of visceral epithelial cells. Treatment of tissue with neuraminidase exposes diffuse reactive sites on the epithelial plasmamembrane. This pattern of localization suggests that gp330 is synthetized by the podocytes, transported to the Golgi where terminal glycosylation occurs [56], ferried to the cell membrane via secretory vesicles, and concentrated into the coated pits [52, 54]. However, it is difficult is to explain the presence of gp330 in multivesicular bodies which have been shown to discharge gp330 into the urinary space [56]. Multivesicular bodies are prelysosomal structures involved in uptake of both intracellular [57] and circulating proteins [58]. It has been proposed that gp330 may be transferred directly from the Golgi to the multivesicular bodies, or that cell surface gp330 may be internalized into multivesicular bodies, or that gp330 produced elsewhere may bind to coated pits, taken up by endocytosis, and subsequently delivered to the multivesicular bodies [59]. The second and the third hypothesis are consistent with a bidirectional cellular transport of gp330. The pattern of localization at the cell surface suggests that this glycoprotein may be a receptor or a ligand [52, 59]. Recent studies report that monoclonal antibodies to gp330 block the adherence of tubular epithelial cells to the basement membrane matrix. Further, gp330 would selectively bind to fibronectin. Taken together these results are interpreted as evidence that gp330 is a fibronectin receptor [60]. In other absorptive epithelia (intestine, epididymis, and embryonic visceral yolk sac) gp330 is also localized in coated pits, Golgi, endoplasmic reticulum and multivesicular bodies [61].

The discovery of gp330 was a stimulus for studies designed to extend the mapping of cell surface antigens in the glomerular capillary wall. Other antigens, isolated from the brush border and shared by glomerular cells were described. It is not certain that all the preparations represent single glycoproteins. The data concerning gp600, for example, suggest that it may be a labile complex of five loosely associated polypeptides, possibly a precursor of gp330 [62]. Another antigen of 108 kD was characterized as a monomer of dipeptidyl peptidase IV [63]. Several investigators [64–67] isolated, alone or in the association with gp330, a 70-90 kD protein present in transport epithelia, in the glomerular endothelium [65–67], and in the majority of normal lymphocytes [68]. gp70-90 is dipeptidyl peptidase IV [67], one of the major hydrolases of the renal brush border and of the gut, probably responsible for the hydrolysis of physiologically active peptides. Besides, dipeptidyl peptidase IV seems to be involved in the fibronectin-mediated spreading of cells on basement membrane matrices [69]. A 140 kD molecule, corresponding to the highly negatively charged coat (polyanion) of glomerular cells has been isolated and called "podocalyxin" [70]. Podocalyxin is evenly distributed on the podocyte plasmalemmal surface [70] but not at the "soles" of the foot processes [71]. Moreover, it is unevenly distributed on the luminal membrane domain of many endothelia, including the glomerular endothelium [72]. Another glycoprotein, with electrophoretic mobility (130 kD) lower than that of podocalyxin was detected by immunohistochemical methods at the base of the foot processes in tissue treated with neuraminidase [73]. Heparan sulphate proteoglycans, different from those present in

the laminae rarae of the glomerular basement membrane, have been shown in the glycocalyx of glomerular epithelial and endothelial cells [74]. A similar localization is characteristic of another 62 kD protein called "podoendin" [75].

c. Antibodies. The nephritogenic potential of antibodies elicited by immunization with each separate component of the tubular brush border has not been established with equal rigor. The best characterized antibodies are those reactive with gp330 [51]. They are consistently eluted from glomeruli of nephritic rats [51, 76], induce subepithelial immune deposits after passive transfer [59, 76], and are present in the sera of rats immunized with Edgington's original brush border preparation [76]. Active immunization with gp330 induces subepithelial immune deposits while proteinuria (200-400 mg/24 hours) is observed in a few rats [76]. Since immunization with Fx1A is associated with glomerular lesions and proteinuria more severe than those occurring after immunization with gp330 [64, 67], it was suggested that crude antigenic preparations stimulate synthesis of multiple nephritogenic antibodies. The most likely candidates to enhance the effect of antibodies to gp330 are antibodies to dipeptidyl peptidase IV (gp70-90), an antigen mainly expressed at the surface of glomerular endothelial cells [54, 66, 67]. First, after injection, or perfusion of rat kidney with rabbit antiserum to anti-rat Fx1A, which contains antibodies to gp70-90, fine granular deposits of rabbit IgG are seen in the glomerular endothelium. The deposits rapidly disappear and, after 8 hours, become detectable at the epithelial side of the basement membrane [77]. The short-lived, complement-fixing, endothelial interaction may contribute to increase the glomerular permeability, thus facilitating the access of anti-gp330 antibodies to the surface of epithelial cells [67]. The hypothesis that anti-endothelial antibody may contribute to the pathogenesis of classical Heymann glomerulonephritis is supported by the observation that when rabbits are injected with antibodies to ACE, immune complexes are initially formed at the level of the glomerular endothelium and subsequently migrate across the basement membrane and aggregate at its epithelial side [78] (see section Glomerular endothelial cells. 1. Lesions mediated by anti-ACE antibodies). Second, subepithelial deposits are observed in rat kidneys perfused ex vivo with polyclonal antibodies reactive with the 90 kD components of Fx1A [79], and transient subepithelial deposits are seen in kidneys of rats injected with an anti-90 kD monoclonal antibody reactive with the brush border and with the plasma membrane of glomerular endothelial and epithelial cells [67, 80]. Third, anti-gp90 antibodies can be eluted from glomeruli of nephritic rats [64, 65]. Fourth, anti-gp70-90 antibodies induce Heymann glomerulonephritis in mice [81, 82] and transient subepithelial immune deposits in rabbits [67]. Other antibodies with nephritogenic potential are those reactive with laminin, fibronectin, and type IV collagen. For reasons that are presently unknown, immunization with Fx1A elicits the synthesis of antibodies which bind to the glomerular basement membrane with a linear pattern and fix complement. Furthermore, injection of anti-Fx1A antibodies absorbed with glomerular basement membrane fails to induce formation of subepithelial deposits as large as those seen in rats injected with the unabsorbed antiserum [83].

Not much is known about the genetic control of susceptibility to develop Heymann glomerulonephritis. LEW, AS, BDV, L/BDV, AS2 and L/AS2 rat strains are highly susceptible, whereas BN, AVN and DA strains are resistant, even after six months of observation and administration of additional adjuvants [84, 85]. These differences may be related either to the expression of relevant antigens on glomerular epithelial cells or to the quality and quantity of antibodies. Concerning the latter issue, it is evident that the activation of autoreactive B cells is the pivotal event in the pathogenesis of the disease. Investigations on the cellular mechanisms of antibody production have been limited to assays with crude brush border fractions and have shown that the autoantibody response is T cell-dependent [86–88] and that suppressor T cells are responsible for resistance [89]. More difficult has been to establish whether specific alterations of T lymphocyte subsets account for different susceptibility among various strains. Well defined differences have not been found suggesting that, if present, these alterations must be of a very subtle nature, possibly related to different suppressor T cell recruitment by the thymus [90]. Interesting results were obtained when LEW-1N kidneys were transplanted into BN rats. The two strains are RT-1 identical ($RT\text{-}1^n$), but differ in an unknown number of weak histocompatibility loci, so that grafts survive without signs of rejection for at least five months. Membranous glomerulonephritis, characterized by anti-brush border antibodies, develops in the renal graft but not in BN native kidney. Antibodies eluted from the graft react with the brush border of LEW-1N kidneys but not with that of BN rats. In contrast, antibodies eluted from glomeruli of LEW rats with classical autoimmune Heymann glomerulonephritis react with the brush border of BN kidney [91]. The experiments show that Heymann autoantibodies and alloantibodies have different specificity, and that LEW-1N and BN kidneys have different susceptibility to develop Heymann glomerulonephritis. It is unknown whether these differences depend upon the quality and quantity of antibodies or upon the expression of relevant antigens at the surface of glomerular epithelial cells. In conclusion, though antibodies to gp330 are indispensable for the development of Heymann glomerulonephritis, full expression of injury probably requires cooperation of polyvalent antibodies and multiple antigen-antibody systems [67, 80, 92].

d. Pathogenesis. Immune deposits are the hallmark of immune complex injury and the understanding of their formation sheds light on the pathogenesis of Heymann glomerulonephritis. Studies have been performed in vitro and in vivo. In vitro it was observed that after interaction at 37°C of divalent polyclonal antibodies to Fx1A with cultured rat glomerular visceral epithelial cells, immune complexes are rapidly redistributed at the cell surface with patching, capping and shedding. Monovalent antibodies fail to induce similar results indicating a need for cross linking. Further, drugs blocking energy production or inhibiting the cytoskeleton prevent redistribution of immune complexes at the cell surface. Polyclonal and most remarkably monoclonal antibody to gp330, recognizing fewer reaction sites, produce similar but weaker effects [93]. In other studies brush border antibodies characterized by prevalent gp330 reactivity convert Heymann antigens from a detergent-soluble, membrane-associated form into an insoluble, actin-associated form [94] in apparent agreement with other results showing that antibodies to gp330 mainly induce internalization of immune

complexes, whereas antibody to podocalyxin and to Fx1A mainly induce immune complex shedding (Camussi et al, unpublished observations). Would these data be valid for the interpretation of the antigen-antibody interaction in vivo the concept that the subepithelial immune deposits result from endocytosis, intracellular lysosomal processing and subsequent secretion into the subepithelial space might be entertained. It must be kept in mind, however, that cultured cells may change polarity of secretion and expression of surface antigens. Moreover, in vitro, Fx1A antibodies recognize antigens, such as podocalyxin and heparan sulphate proteoglycans, at the free surface of the podocytes. By way of contrast, in vivo subepithelial immune deposits result exclusively from interaction of circulating antibodies with antigens at the base of the foot processes, or in other layers of the capillary wall proximal to the circulation.

Studies of passive Heymann glomerulonephritis only partially confirm the results obtained in vitro. Kerjaschki and Farquhar [52] and Allegri et al [92] showed that formation of immune deposits requires polyclonal antibodies to gp330 able to cross link extensively the antigens expressed at the surface of epithelial cells. Besides, epitope specificity was recognized as an important characteristic of nephritogenic antibodies because not all antibodies to gp330 are able to induce formation of subepithelial deposits. It was proposed that nephritogenicity may require reactivity with epitopes normally not expressed in vivo [67]. Chlorpromazine, a drug that blocks calmodulin and protein kinase C and decreases plasma membrane fluidity [95], inhibits shedding of immune complexes and formation of immune deposits. This rapid and potent action was ascribed to the effect of the drug on the cytoskeleton [96]. Important information was obtained by Kerjaschki, Miettinen and Farquhar [59] in studies concerning the initial formation of subepithelial immune deposits in rats injected with divalent polyclonal antibodies to gp330. After crossing the glomerular basement membrane the antibodies bind to clathrin-coated pits at the base of the foot processes. The resulting immune complexes are shed into the lamina rara externa and, after 15 minutes, form morphologically-recognizable immune deposits which remain in contact with coated pits. The immune deposits, containing both gp330 and the heterologous antibody, grow in size presumably by repeated cycles of immune complex formation and shedding. In time, both antigen and antibody are demonstrated at some distance from the ectodomain of gp330 and the immune deposits are eventually driven by hydrodynamic forces into the area of the slit diaphragm. Continuous growth probably requires de novo synthesis of gp330 molecules, transport of gp330 to the cell surface via secretory vesicles and insertion of gp330 into the coated pits at the base of the foot processes. The observation that a coated pit is always in contact with immune deposits might result from movement of the foot processes along the outer surface of the basement membrane and represent an expression of delivery of gp330 to the cell surface. Here gp330 would bind to antibodies present in excess in the immune deposits which would remain firmly attached to the basement membrane by a covalent link similar to that induced by free oxygen radicals [97] or by interaction with the C3b receptors of laminin [98]. If this interpretation is correct gp330 would behave like a "secreted" antigen rather than a classical cell surface receptor and there is indeed evidence that the synthesis of gp330 is increased in the ergastoplasm of the podocytes when specific antibody interact with the cell surface [54]. This issue is germane to the function of the coated pits, generally recognized as structures specialized in receptor-mediated endocytosis [10]. It is presently unknown whether the coated pits of the podocytes in rats genetically susceptible to develop Heymann glomerulonephritis have some internalization-defective mutation [99]. Such an abnormality would be analogous to the deletion of exons encoding transmembrane and cytoplasmic domains of LDL receptors. These truncated receptors are largely secreted, rather than internalized with ligands [100]. In Heymann glomerulonephritis, however, gp330 interacts with an antibody, not with a physiologic ligand. A second possibility is that the coated pits of podocytes may function like those of the lactating mammary gland, which are involved in a well defined secretory pathway [101]. A third contention proposes that gp330 is a ligand physiologically internalized at the level of the coated pits [59]. In Heymann glomerulonephritis crosslinking antibodies would promote shedding of the ligand from the coated pits. This interpretation, however, does not account for the large amount of gp330 apparently synthesized by the podocytes [52, 54]. These crucial aspects of the pathogenesis of Heymann glomerulonephritis will be better understood when the techniques of molecular biology are brought to bear to establish the amino acid sequence of the gp330 molecule and to clarify its function. Thus far, immunohistochemical studies of passive Heymann glomerulonephritis as accurate as those performed by Kerjaschki, Miettinen and Farquhar [59] have not been performed with antibodies reactive with other cell surface antigens of the glomerular capillary wall.

In Heymann glomerulonephritis the formation of immune deposits does not directly induce tissue injury. Rather, damage occurs because the complement system is activated [102]. The demonstration that deposits of rat C9 and complement membrane attack complex neoantigens are present in glomeruli of rats with Heymann glomerulonephritis suggest that the membrane attack complex (C5b-C9) is directly responsible for epithelial cell injury and proteinuria [103–105]. This hypothesis is confirmed by experiments showing that increased protein excretion and glomerular epithelial cell damage does not occur when isolated rat kidneys are perfused with Fx1A antibodies and C8-deficient human plasma or C6-deficient rabbit serum [106, 107]. Studies performed with cultured glomerular epithelial cells confirm that cell lysis depends on insertion of C5b-C9 into the epithelial plasma membrane. It was also found that C5b-C9 is inserted at sites different from antibody-binding sites and that, eventually, it is shed into the supernatant fluid [108], a reparative process described in other types of nucleated, not completely lysed, cells [109]. Parallel studies were performed in renal tissue obtained at sequential intervals of time from rats with passive Heymann glomerulonephritis. Deposits of C5b-C9 are first detected between the foot processes and the basement membrane. By day 3 C5b-C9 is internalized into multivesicular bodies and exocytosed into the urinary space. Concomitantly, C5b-C9 is detected in degraded form in the urine [110]. These observations are discussed in greater detail by Salant et al elsewhere in this issue. gp330 and heterologous IgG are not seen in the multivesicular bodies, indicating that immune com-

plexes and the lytic system of complement follow different pathways [110]. The observations that in vitro C5b-C9 appears shed from the cell surface [108], whereas in vivo it is internalized, shows, again, that discrepant results are generated by the two experimental approaches.

The discovery that the main pathogenetic mechanism of Heymann glomerulonephritis is an in situ formation of immune deposits does not completely exclude the possibility that circulating immune complexes contribute to injury. This hypothesis is based on the reports of immune complexes in the sera of rats with active Heymann glomerulonephritis [111], and of gp70-90 in the sera of normal rats [66]. An antibody reactive with a 45 kD glomerular antigen, but not with the brush border, would have the properties of an anti-idiotypic antibody to Fx1A and would be generated by immunization with a not defined component of the brush border. Such an antibody would have a pathogenic role in the development of lesions because it is concentrated in glomeruli of nephritic rats, and appears in the serum in concomitance with proteinuria [112, 113].

Another remarkable effect of brush border antibodies is their ability to induce malformations in the offspring when given to pregnant rats during the organogenetic period [114]. This important issue has been recently re-examined. It was found that injection in pregnant rats of a rabbit anti-rat brush border gp340 kD glycoprotein serum induces abnormal embryonic development, especially frequent anaphtalmia, fetal retardation and embryonic death. In vivo the antibodies are bound to the visceral yolk-sac endodermal cells and the embryonic endoderm [115, 116]. Rabbit antiserum to embryonic visceral yolk-sac brush border exhibits an immunoprecipitin band in the 330 region and, upon injection into rats, induces a classical Heymann glomerulonephritis [116]. Other studies show that the teratogenic antibodies are reactive with a 280 kD protein present in intermicrovillar areas, coated pits of proximal tubular brush border, and epithelial cells of the visceral yolk sac. Antibodies to gp330 are devoid of similar teratogenic effects. Since the 280 kD protein is localized in clatrin coated pits it is possible that alterations of a specific constituent of the receptor-mediated endocytic system may induce fetal malformations [117].

e. Heymann-like glomerular injury in other species. The co-expression of some antigens in tubular and glomerular cells is not a prerogative of rats susceptible to Heymann nephritis. The phenomenon occurs in other species. However, only mice and, to a lesser extent, rabbits co-express antigens relevant to the formation of glomerular subepithelial immune deposits. In mice passively immunized with heterologous anti-mouse Fx1A the antibody persists in the glomerular capillary wall for a time and in amount sufficient to generate a proteinuric autologous phase [81]. The anti-Fx1A serum recognizes the 90 kD dipeptidyl peptidase IV diffusely distributed on the plasma membrane of glomerular epithelial and, in lesser degree, endothelial cells. The antiserum also reacts with rat gp90 and gp330, binds to the coated pits of rat glomerular epithelial cells, and induces Heymann glomerulonephritis in this species [82]. Thus, the mouse does not express gp330 but, nevertheless, subepithelial immune deposits develop by interaction of polyclonal antibodies with an antigen apparently not localized in the coated pits [67].

In the rabbit, monoclonal antibodies to homologous brush border 100 kD dipeptidyl peptidase IV or endopeptidase bind to antigens diffusely localized in the plasma membranes of glomerular epithelial cells. When rabbits are injected with these antibodies transient subepithelial immune deposits are seen comparable to those present in rats injected with antibodies to gp70-90 [67]. Heterologous rabbit Fx1A antiserum produces in the rabbit transient subepithelial immune deposits and proteinuria. In contrast, rabbits actively immunized with homologous brush border preparations develop a tubular immune complex disease but not glomerulonephritis, indicating that brush border antibodies do not recognize cross-reactive antigens in glomerular epithelial cells [118]. A different pathogenetic mechanism of glomerular injury was proposed for rabbits injected with guinea pig anti-brush border sera. The antibodies would release antigens from the brush border with consequent "implantation" in glomeruli, with formation of circulating immune complexes, or both [119]. Dogs seem to be refractory to Heymann-like nephritis [120].

2. Lesions mediated by a monoclonal antibody against SGP-115/107. Monoclonal antibodies are generally, relatively inefficient in inducing tissue damage. An apparent exception is a particular mouse monoclonal antibody of the non-complement fixing IgG_1 class (K9/9) directed against a sialo-glycoprotein with a molecular weight of 115 and 107 kD (SGP-115/107) [121]. SGP-115/107 is found in the surface of epithelial cells of various organs. K9/9 injected into rats binds to glomerular visceral epithelial cells and causes morphological changes of these cells consisting of focal disappearance of the epithelial foot process system, vacuole formation, microvillous transformation, and focal retraction of podocytes. In contrast to Heymann nephritis, granular, subepithelial immune deposits are not found in the glomerular capillary wall in this model. This may be due to the fact that SGP-115/107 is non-redistributable or, more likely, to the fact that the monoclonal antibodies provide for insufficient cross linkage of SGP-115/107 [93]. Animals that receive K9/9 together with complete Freund's adjuvant develop transient proteinuria. The need for non-specific stimulation of the immune system may reflect the synergistic participation of cytokines in the induction of this functional abnormality [121]. The K9/9 associated glomerular pathology appears to be complement- and leukocyte-independent.

It is remarkable that monoclonal antibodies that share with K9/9 a reactivity with SGP-115/107 but bind to different epitopes, fail to generate visceral epithelial cell lesions in vivo [122]. Thus, this novel model of glomerular injury depends on the interaction of antibody with a specific epitope of a cell surface antigen. The model clearly illustrates that it is not just the antigen specificity, but also the epitope specificity that determines the biological effect of antibody binding.

Glomerular endothelial cells

1. Lesions mediated by anti-ACE antibodies. Although easily detected in other vascular beds, routine immunohistological techniques fail to reveal ACE on the surface of rabbit glomerular endothelial cells [78]. Evidence for the presence of ACE on glomerular endothelial cells comes from experiments in which rabbit kidneys were perfused ex vivo with goat anti-ACE antibodies [78]. The results show a seven times higher glomer-

ular binding of anti-ACE IgG than of non-immune IgG, and granular deposits of goat IgG on the glomerular endothelium. When rabbits are given goat anti-ACE antibodies for four days, granular deposits of goat IgG are seen on the glomerular endothelium on day one; from day three to 24 the gradual development of subepithelial deposits containing goat IgG and rabbit IgG and C3, and located mainly in the filtration slits, is observed. The interpretation has been that immune complexes formed after in vivo binding of specific antibodies with ACE are shed from the endothelium and then, under the influence of hydrodynamic forces and possibly the release of vasoactive substances from the endothelium [123], relocate at the epithelial side of the glomerular basement membrane [78].

That circulating ACE-anti-ACE antibody complexes may contribute to the subepithelial immune deposits in the glomerular capillary wall is suggested by the finding that, when anti-ACE antibodies are administered to rabbits made proteinuric by repeated injections of cationic bovine serum albumin [124], not only goat IgG and rabbit IgG and C3, but also ACE can be demonstrated in these deposits [78]. It is reasonable to propose that, according to classical concepts [125], a local increase in permeability facilitates the localization of immune complexes from extrarenal vascular beds, such as of the lung, richly endowed with ACE.

2. Lesions mediated by transplantation antibodies. It is beyond the scope of this review to discuss in detail humorally-mediated kidney transplant rejection, so only a few comments will be made. Most clinically relevant alloantigens, such as MHC Class I and II, blood group, and endothelial-monocyte antigens, can be demonstrated on kidney endothelial cells [126–128]. Like other surface proteins, transplantation antigens are under proper conditions subject to antibody-mediated redistribution and endocytosis [6, 7]. Furthermore, their expression is subject to modulation by cytokines [129, 130].

Transplantation antibodies not only are responsible for hyperacute kidney allograft rejection, but are also thought to participate in the acute and chronic rejection processes. Cross linkage of transplantation antigens may be an essential first step in the activation of effector mechanisms, such as the complement system, or in the release of vasoactive substances, such as platelet activating factor and arachidonic acid derivatives [131].

Donor-specific alloantibodies administered at the time of kidney transplantation may enhance rather than shorten graft survival [132]. The balance between enhancement and rejection may depend on the class, affinity, and specificity of the transplantation antibodies. In the absence of immunohistological observation, one can only speculate on a role for antibody-mediated antigenic modulation in the phenomenon of enhancement.

Mesangial cells

Lesions mediated by anti-thymocyte antibodies. T cell epitopes have been shown on the surface of rat mesangial cells [133–135]. When injected into rats, heterologous anti-thymocyte antibodies bind, together with C3 and late acting complement components, to the mesangial cells; after four days the immune reactants are no longer detectable in glomeruli [79, 136, 137]. Within one hour of the binding severe mesangial cell lesions, including lysis, are observed. In the days that follow extensive mesangiolysis and endo- and extra-capillary cell proliferation become apparent. After several months the mesangial pathology resolves without scar formation. The antibody-mediated mesangial cell injury is complement-dependent, but neutrophil-independent [136, 137].

This mesangiopathy and Heymann nephritis share a common pathogenesis: both conditions result from the reaction of antibodies with plasma membrane antigens and both are complement-dependent. However, in contrast to the mesangiopathy, in Heymann nephritis persistent immune deposits are formed and cell lysis is not an obvious feature, although lysis of target cells does occur in vitro [108]. These differences serve to emphasize the importance of local circumstances and of the cell types involved in the eventual outcome of injury similarly induced at various tissue sites.

Tubular cells

1. Heymann nephritis. Once rats actively immunized with Fx1A become proteinuric, autoantibodies passing into the urinary space bind specifically to antigens on the brush border of proximal tubules [138, 139]. Concomitantly these rats develop lesions of the proximal tubules consisting of swelling and fragmentation of the brush border with accumulation in tubular lumina of numerous plasma membrane vesicles, disappearance of apical vesicles, degenerative changes, and cell proliferation [139, 140]. Correlating with the eventual decrease in the titer of circulating anti-brush border antibodies, there is a morphological recovery of the proximal tubules, although functional abnormalities persist [141]. Proof for the cytotoxic action of anti-brush border antibodies comes from the finding that the proximal tubular lesions seen in active Heymann nephritis can be passively transferred with these antibodies [142]. To produce tubular pathology both antigen cross linking and cytoskeleton activation are required [143]. However, at variance to what has been observed for glomerular epithelial cells [102–108], the damaging effect of Heymann antibodies on proximal tubular cells appears to be complement-independent [144].

Rats with active Heymann nephritis not only show subepithelial glomerular capillary wall immune deposits, but also subepithelial proximal tubule immune deposits [138, 139]. These latter deposits, which contain gp330, initially form in contact with the basal part of the plasma membrane of the epithelial cells and can be seen between the basal infoldings [139]. It is conceivable that they result from the local interaction of antibodies, which have passed the tubular basement membrane from the interstitium, with Heymann antigens sparsely expressed on the basolateral membrane of proximal tubular cells. Alternatively, one might propose that antigen-antibody complexes transported from the apical part are released at the base of proximal tubular cells. The tubular deposits are only temporarily present and they disappear in parallel with the circulating autoantibodies. The contrasting persistence of the glomerular immune deposits in Heymann nephritis is consistent with a role for local factors in the turnover and clearance of immune deposits [145]. The mechanism of removal of tubular immune deposits is not known but may involve their solubilization by local antigen excess or the crossing of small immune deposit fragments of the tubular basement membrane with subsequent disposal via renal lymphatics [145, 146].

2. Lesions mediated by anti-ACE antibodies. ACE is expressed on proximal tubular cells, densely on the apical and, much less, on the basolateral plasma membrane [147]. Goat anti-Ace antibodies injected intravenously into rabbits will leave peritubular capillaries and reach the base of proximal tubules where they bind to ACE on the basolateral membrane (Fukatsu et al, unpublished observations). ACE-antibody complexes then appear to be shed in the basolateral compartment, forming small immune deposits consisting of ACE, goat IgG, and rabbit C3. The tubular deposits are only transient and they disappear when anti-ACE antibodies are no longer available. The extra-vascular location of the immune complex aggregates might explain the mild or absent inflammatory reaction [148].

In rabbits made proteinuric, anti-ACE antibodies also bind, together with complement, to the brush border of proximal tubules. However, unlike Heymann antibodies, anti-ACE antibodies do not inflict significant damage to the apical part of proximal tubules.

Secreted antigens

Glomerular visceral epithelial cells

Mercuric chloride-induced glomerulonephritis. Mercuric chloride administration to rabbits or BN rats causes a biphasic autoantibody-mediated glomerulonephritis [149, 150]. In the early weeks after the start of injections of mercuric chloride a linear deposition of IgG along the glomerular basement membrane is seen. Later in the course of the disease the distribution of IgG assumes a discrete granular pattern and electron dense deposits are present at the epithelial side of the glomerular basement membrane. The nephropathy now has the characteristics of a membranous nephropathy. Studies of sera reveal antibodies to laminin and type IV collagen [151, 152], which are major components of the glomerular basement membrane. Antibodies with the same specificity can be eluted from kidneys with linear as well as from kidneys with granular immune deposits. The eluates from nephritic kidneys react with antigens synthesized and secreted by cultured rat glomerular visceral epithelial cells. The pattern of reaction of the eluted antibodies is similar to that observed with anti-laminin and anti-type IV collagen antibodies. Absorption of the eluates with both laminin and type IV collagen blocks the reactivity with the cultured cells. Binding of the eluates with the apical surface of the cultured cells, as is the case with Heymann antibodies, is not found [152]. These data are consistent with the hypothesis that prolonged, continuous interaction of antibodies with basement membrane antigens secreted by visceral epithelial cells may contribute to the formation of granular immune deposits in the subepithelial part of the glomerular basement membrane [152]. According to this concept, both phases of mercuric chloride glomerulonephritis are different expressions of the same underlying process. One of the several alternative explanations is that the granular immune deposits in mercuric chloride nephropathy are derived from circulating immune complexes [153]. However, against such an event and in favor of in situ immune deposit formation is the finding in rabbits and rats treated with mercuric chloride of granular immune deposits in organs and structures not especially involved in filtration and generally not affected by injury resulting from entrapment of circulating immune complexes [154, 155].

DZB rats receiving mercuric chloride develop membranous glomerulonephritis, which, unlike in BN rats, does not seem to be preceded by a phase of linear localization of IgG along the glomerular basement membrane [156]. In sera and kidney eluates, only antibodies against laminin can be demonstrated. By immunoelectron microscopy, the binding of eluted antibodies to normal rat kidney is restricted to the cell membrane of glomerular and tubular epithelial cells adjacent to the basement membrane. In this model a pathogenic role is proposed for autoantibodies against a cell-binding epitope on the laminin molecule [156].

Several other animal models further support the notion that antibodies to basement membrane components may be involved in granular immune deposit formation in glomeruli: anti-laminin and type IV collagen antibodies have been implicated in the immune complex glomerulonephritis associated with experimental trypanosomiasis in the rat [157, 158]; subendothelial and mesangial immune deposits are generated in mice actively immunized with purified laminin [159]; and, lastly, rabbits immunized with autologous basement membrane develop linear as well as granular immune deposits along the glomerular basement membrane [160].

Tubular cells

1. Lesions mediated by anti-Tamm-Horsfall protein antibodies. Tamm-Horsfall protein is synthesized in renal distal tubules and excreted in normal urine. It is identical to the immune suppressive glycoprotein uromodulin and appears to specifically bind to and regulate the activity of certain cytokines including IL-1 and TNF [161]. Tamm-Horsfall protein is located at the surface of the luminal and basolateral membrane and in apical vesicles and the Golgi complex of cells of the thick ascending limb of the loop of Henle and the distal convoluted tubule [162–164]. Rats actively or passively immunized with Tamm-Horsfall protein develop granular deposits composed of this protein and of rat IgG and C3 in between the basal infoldings at the epithelial side of the basement membrane of the thick ascending limb of the loop of Henle [165, 166]. In immunized mice, similar deposits are also found along distal convoluted tubules [164]. The presence of circulating antibodies to Tamm-Horsfall protein is required to prevent a rapid clearance of the tubular immune deposits [145, 146]. There is no inflammatory reaction following the immune deposit formation [164, 165]. Inflammation at the base of the ascending thick limb and scarring are seen in rabbits immunized with Tamm-Horsfall protein [167]. The rabbit model differs from that established in the rat and mouse in that immunoglobulin deposition is not a feature [167].

Antibodies to Tamm-Horsfall protein injected into rats artifically made proteinuric bind to the luminal surface of the cells of the thick ascending limb of the loop of Henle, an event followed by the appearance of large luminal electron-dense deposits [168]. Deposits in the lumen of proximal tubules are not observed in Heymann nephritis [139]. Similar, however, is the observation of increased mitotic activity in the affected tubular segments [140, 168]. Antibody reactions with Tamm-

Horsfall protein are discussed more extensively by Wilson elsewhere in this issue.

2. Tubulointerstitial nephritis in rabbits mediated by autoantibodies to a proximal tubular 70 kD antigen. Rabbits receiving repeated kidney allografts [169] or actively immunized with homologous glomeruli-free kidney cortex [170, 171] or homologous Fx1A [118], reveal extensive peritubular fibrosis and tubular atrophy together with a mild infiltration of the interstitium with mononuclear leukocytes. Some of the rabbits have glucosuria and aminoaciduria. Granular deposits of IgG and C3 can be demonstrated at the epithelial side of the basement membrane of proximal tubules. Glomerular deposits are absent. The serum and kidney eluates of the animals contain antibodies reactive in vitro with normal proximal tubular cells. In addition, these antibodies bind to the granular deposits in diseased kidneys [172]. Passive transfer of serum of immunized rabbits to normal rabbits results in focal deposition of IgG along tubular basement membranes in a pattern identical to that seen in the donors. Immunoelectron microscopy using eluates of nephritic kidneys shows the responsible antigen to be located in normal rabbits on the brush border and apical vesicles of proximal tubules. The antigen has an apparent molecular weight of 70 kD (Fukatsu et al, unpublished observations). Observations made in this rabbit model of tubulointerstitial nephritis led to the original formulation of the hypothesis that granular immune deposits in tissues may form by the local reaction of circulating antibodies "leaking" from or secreted by cells surrounded by basement membrane [171]. Species differences in epitope recognition may explain why in rabbits the administration of heterologous anti-rabbit Fx1A antibodies causes glomerular but not tubulointerstitial lesions [118].

Arterial muscle cells

SL/Ni mice spontaneously develop a systemic arteritis that also involves the kidney [173]. The arteritis is characterized by segmental fibrinoid necrosis of the vessel wall and dense perivascular inflammatory cell infiltrates, lesions not unlike those seen in patients with periarteritis nodosa. Just before or at the time of onset of the arteritis, budding of a large number of murine leukemia viral C-type particles from the plasma membrane of muscle cells of the arterial media is observed. The earliest injury, consisting of degeneration and necrosis of medial muscle cells, may be mediated by the binding of complement-fixing antibodies to gp70 of the C-type particles on the cell surfaces [174]. In a later phase of the disease, granular deposits of immunoglobulins, complement, and viral antigens accumulate in the wall of affected arteries. These immune deposits, presumably derived from circulating immune complexes, may amplify the arterial injury.

Planted antigens

Lesions mediated by anti-Helix pomatia lectin antibodies

In normal glomeruli, Helix pomatia lectin binds especially to the glycocalyx of visceral epithelial cells between the base of the foot processes and the basement membrane [175]. An increase in glomerular lectin binding sites is observed after neuraminidase treatment. The increase is striking for the surface of podocytes facing the urinary space and for the surface of endothelial cells. Rat kidneys first perfused ex vivo with neuraminidase and then with Helix pomatia lectin reveal the lectin exclusively on the glomerular endothelium [176]. In rats given subsequently an intravenous injection of anti-lectin antibodies, immune complex aggregates form on the endothelial cell surface. With time, these deposits move from the endothelium to a subepithelial location [176]. The events are accompanied by mild proteinuria. These data provide further evidence that antibody-endothelial cell surface interaction may lead to the formation of subepithelial immune deposits in the glomerular capillary wall.

The significance of animal models for human kidney pathology

Despite all the studies in laboratory animals, the understanding of the etiology and pathogenesis of the various human nephropathies is still very limited. However, concepts derived from experimental immunopathology at least provide leads helpful in the search for the origin of the nephropathies affecting our patients. A case in point is membranous glomerulonephritis, the most frequent cause of nephrotic syndrome in adults. There is a remarkable similarity between the glomerular abnormalities in human membranous glomerulonephritis and those in Heymann nephritis: in both, non-inflammatory, conditions immune deposits are strictly limited to the subepithelial space and seem to initiate the same basement membrane changes, which include the characteristic "spikes". An attractive proposition is that most if not all forms of human membranous glomerulonephritis result from the local binding of autoantibodies with antigens either intrinsic to or planted on the surface of cells of the glomerular capillary wall [1, 2]. However, unlike Heymann nephritis, injury of proximal tubules associated with local deposits of immunoglobulins is not seen in human membranous glomerulonephritis. This would suggest that the antigens responsible for membranous glomerulonephritis in man are not shared by the glomerular capillary wall and the brush border of proximal tubules [177–179]. This notion is supported by the following observations: First, although there are a few descriptions of patients with Heymann-like nephritis in the literature [180–186], attempts to detect brush border antigens in the glomerular deposits of human membranous glomerulonephritis have generally been unsuccessful [187–190]. Second, in normal human kidneys a plasma membrane protein of 400 kD, cross reactive with the Heymann antigen gp330, is present on proximal tubular but not on glomerular epithelial cells [178]. Third, an effort to induce a Heymann-like nephritis in non-human primates has failed so far. Monkeys actively or passively immunized with human Fx1A develop a tubular but not a glomerular immune complex disease [191]. In addition, in vitro perfusion of normal human kidney with rabbit anti-human brush border antibodies does not result in glomerular immune deposits [191]. Thus it seems unlikely that normal human brush border expresses antigens relevant for the induction of in situ immune complex formation in primate glomeruli.

It has been reported that glomerular eluates from patients with idiopathic glomerulonephritis react with a 90 kD human renal glycoprotein and bind to glomerular immune deposits in 50 percent of patients with the disease tested [192]. A pathogenesis similar to that proposed for experimental mercuric chloride-induced glomerulonephritis [152] may be considered for membranous glomerulonephritis occasionally following au-

toimmune anti-glomerular basement membrane nephritis [193] or occurring in association with certain drugs [194–198].

One of the many problems encountered in the identification of the pathogenic antigens in human membranous glomerulonephritis is that autoantibodies, presumably present at the onset, may no longer be in the circulation at the time that patients have developed clinical symptoms, making serological studies unproductive. The search for responsible antigens should continue making use, among other means, of monoclonal antibodies to defined antigens on human glomerular cells. Intensified studies of drug-induced or cancer-associated membranous glomerulonephritis, may also prove to be rewarding.

Sera of patients with systemic lupus erythematosus and kidney eluates of lupus-prone mice [199] contain antibodies reactive with so-called "lupus associated membrane protein" (LAMP), which is distributed on the surface of a variety of cells, including glomerular epithelial cells. LAMP is located in clathrin coated pits on these latter cells [200]. Interestingly, LAMP is recognized by a monoclonal antibody to dsDNA. These data raise the possibility that in addition to deposition of circulating immune complexes, in situ formation of immune complexes may account for the subepithelial immune deposits in the kidneys of patients with lupus membranous glomerulonephritis [200].

Tubular immune deposits are frequently noticed in lupus nephritis [201]. Their widespread distribution suggests that they arise from circulating immune complexes rather than from in situ formed immune complexes. In long functioning human renal allografts, tubular deposits of immunoglobulins and complement are sometimes found in a pattern reminiscent of that seen in rabbits receiving multiple renal allografts [169, 202]. The pathogenesis of these human lesions is unknown.

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